

## In Vitro Antibacterial Activity of Cefoperazone (T-1551), a New Semisynthetic Cephalosporin

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Cefoperazone, a new semisynthetic cephalosporin, has a broad spectrum of antibacterial activity. It is as active as cefazolin and cefamandole against gram-positive bacteria and is more active than cefazolin and cefamandole against such gram-negative bacilli as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* species, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Enterobacter cloacae*, and *Serratia marcescens*. The superiority of cefoperazone over cefazolin and cefamandole with respect to activity against *P. aeruginosa* by more than 200-fold was especially remarkable. As with other  $\beta$ -lactam antibiotics, there was only a small spread between the minimum inhibitory concentrations and the minimum bactericidal concentrations of cefoperazone and a significant decrease in activity with an increase in inoculum size. Activity was not altered significantly by the addition of human serum to the test medium. Cefoperazone is relatively stable to hydrolysis to  $\beta$ -lactamases produced by gram-negative bacteria. Relative rates of hydrolysis of cefoperazone by cephalosporinases are 7.0 to 0.01, with reference to cephaloridine hydrolysis (base, 100). Cefoperazone is also more stable than penicillin G and cephaloridine to various types of penicillinases.

Cefoperazone (T-1551) (CPZ), the sodium salt of 7[D(-)- $\alpha$ -(4-ethyl-2,3-dioxo-1-piperadine-carboxamide)- $\alpha$ -(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1*H*-tetra-*zol*-5-yl)thiomethyl]-3-cephem-4-carboxylate (Fig. 1), has a broader spectrum of activity than related cephalosporins, including cefamandole (CMD) and cefazolin (CEZ) and is significantly active against *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Enterobacter cloacae*. The current report summarizes our observations on the in vitro antibacterial activity of this new cephalosporin and its stability to  $\beta$ -lactamases.

### MATERIALS AND METHODS

**Antibiotics.** CPZ was synthesized in the Research Laboratory of Toyama Chemical Co., Ltd., Toyama, Japan. CMD, CEZ, cephalothin, cephalixin, cephaloridine, carbenicillin (CB-PC), ampicillin, penicillin G, and gentamicin were obtained commercially.

**Strains.** The strains of gram-positive and gram-negative bacteria used in this study were isolated from clinical materials in Japan in 1972 to 1977 and have been maintained since isolation in the Reference Laboratory of Resistant Bacteria, School of Medicine, Gunma University.

**Media.** The heart infusion agar and brain heart infusion broth were products of Eiken Chemical Co., Ltd., and Difco Laboratories, respectively. Other media prepared locally included: peptone broth (10 g of polypeptone, 5 g of NaCl, and 1,000 ml of distilled

water), medium B [2 g of yeast extract, 10 g of polypeptone, 8 g of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , 2 g of  $\text{KH}_2\text{PO}_4$ , 1.2 g of  $(\text{NH}_4)_2\text{SO}_4$ , 2 g of glucose, and 0.4 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  in 1,000 ml of distilled water], and nutrient broth (10 g of beef extract, 12 g of polypeptone, and 5 g of NaCl in 1,000 ml of distilled water). Peptone broth for the preculture of *P. aeruginosa* strains contained 0.3%  $\text{KNO}_3$  to yield a homogeneous culture.

**Determination of MICs.** Minimum inhibitory concentrations (MICs) were determined by a serial dilution technique. Overnight cultures of test strains in peptone broth were diluted to a final concentration of about  $10^6$  cells per ml, and one loopful (0.005 ml) of each culture was spread on the heart infusion agar plates with an inoculator (Microplanter, Sakuma, Tokyo). MICs were determined after overnight incubation at 37°C. The effects of inoculum size on MICs were examined by a serial dilution technique via which the heart infusion agar plates were inoculated with 0.005 ml of cultures diluted to about  $10^4$ ,  $10^6$ , and  $10^8$  cells per ml. The effects of human serum on MICs were investigated by a serial dilution method with the heart infusion agar containing 0, 10, and 40% pooled human serum (Consera, Nissui Pharmaceutical Co., Ltd., Tokyo).

**Bactericidal activity.** Correlation between the MIC and the minimum bactericidal concentration (MBC) was examined as follows. An overnight culture of each strain in nutrient broth was appropriately diluted with the same medium and inoculated in nutrient broth containing serial twofold dilutions of antibiotics to a final concentration of  $10^5$  cells per ml. MICs were determined after incubation for 18 h at

37°C. One loopful (0.005 ml) of each culture tube, after determination of MICs, was seeded onto antibiotic-free heart infusion agar plates and incubated overnight at 37°C. The lowest concentration of antibiotic that inhibited visible growth on these plates was recorded as the MBC. For evaluation of bactericidal activity, an overnight culture in nutrient broth was diluted to  $10^4$  cells per ml with the same fresh medium and incubated with shaking at 37°C. After growth to about  $10^5$  cells per ml, antibiotics were added to the cultures, and numbers of viable cells were counted at 2-h intervals.

**Stability to  $\beta$ -lactamase.** A 20-ml preculture of each strain in brain heart infusion broth was inoculated in 200 ml of medium B and shaken for 3 h at 37°C. Cephalosporinase-producing strains, except

*Escherichia coli* GN5482, were supplemented with penicillin G as an inducer at one-eighth to one-half the MIC and incubated for a further 2 h at 37°C with shaking. Cells were harvested by centrifugation, washed once with 0.1 M phosphate buffer (pH 7.0), and suspended in 5 ml of the same buffer. The cell suspensions were treated by an ultrasonic disruptor for 5 min at 0°C and centrifuged at 4°C for 30 min at 12,000 rpm. The resulting supernatants were used as the crude enzymes.  $\beta$ -Lactamase activity was determined by direct spectrophotometry (10, 13). The reaction mixture consisted of 3 ml of a 100  $\mu$ M substrate solution in 0.05 M phosphate buffer (pH 7.0) and 50  $\mu$ l of enzyme solution. The specific activity of  $\beta$ -lactamase was expressed as micromoles of substrate hydrolyzed per minute per milligram of protein. Protein concentrations in the crude enzymes were determined by the method of Lowry et al. (5). Substrate specificity was expressed as the relative rate of hydrolysis of substrates, taking the absolute rate of penicillin G hydrolysis in penicillinase and cephaloridine hydrolysis in cephalosporinase as 100.

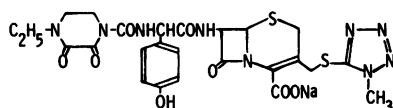


FIG. 1. Chemical structure of CPZ.

TABLE 1. Activity of CPZ against clinical isolates of bacteria

Organism	No. of organisms	Antibiotic	Drug concn ( $\mu$ g/ml)		
			MIC <sub>50</sub>	MIC <sub>75</sub>	MIC <sub>90</sub>
<i>E. coli</i>	200	CPZ	0.19	0.77	3.43
		CMD	1.10	2.09	6.71
		CEZ	1.46	2.68	5.87
<i>K. pneumoniae</i>	100	CPZ	0.21	1.05	5.32
		CMD	1.56	7.34	15.3
		CEZ	1.81	3.00	6.64
<i>P. aeruginosa</i>	200	CPZ	3.60	5.46	12.7
		CMD	>100	>100	>100
		CEZ	>100	>100	>100
		CB-PC	38.5	72.8	>100
<i>S. marcescens</i>	200	CPZ	4.40	45.8	96.0
		CMD	50.0	>100	>100
		CEZ	>100	>100	>100
Indole-positive <i>Proteus</i>	200	CPZ	1.80	3.52	7.57
		CMD	18.4	100	>100
		CEZ	>100	>100	>100
Indole-negative <i>Proteus</i>	100	CPZ	0.76	1.09	1.72
		CMD	1.54	3.05	>100
		CEZ	4.90	6.80	83.1
<i>C. freundii</i>	52	CPZ	0.47	4.00	12.1
		CMD	1.31	6.29	>100
		CEZ	18.0	>100	>100
<i>E. cloacae</i>	200	CPZ	0.16	0.62	30.3
		CMD	3.60	50.0	>100
		CEZ	>100	>100	>100
<i>S. aureus</i>	100	CPZ	1.20	1.75	2.64
		CMD	0.33	0.51	0.70
		CEZ	0.21	0.32	0.40

## RESULTS

**Antibacterial activity.** The antibacterial activity of CPZ against gram-positive and gram-negative bacteria was compared with those of CMD (4, 14), CEZ (6, 8, 9), and CB-PC (1, 3). Antibacterial activity of the drugs against all species tested is shown by the concentrations of the drugs required to inhibit the growth of 50, 75, and 90% of the total number of tested strains, that is, the MIC<sub>50</sub>, the MIC<sub>75</sub>, and the MIC<sub>90</sub>, respectively (Table 1). The peaks of the MIC distributions of CPZ, CMD, and CEZ against *Staphylococcus aureus* were located at 1.56, 0.39, and 0.39 µg/ml, respectively, and CPZ was less active against *S. aureus* than were CMD and CEZ. CPZ showed high antibacterial activity against *Enterobacteriaceae*. MIC<sub>75</sub> values of CPZ against *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* were 0.71, 1.05, and 1.09 µg/ml, respectively, and CPZ was over three times more active than CMD and CEZ against these species. With indole-positive *Proteus* species and *Citrobacter freundii*, MIC<sub>90</sub> values of CPZ were 7.57 and 12.1 µg/ml, respectively, whereas those of CMD and CEZ were more than 100 µg/ml. CPZ was also highly active against *P. aerugi-*

*nosa*, *S. marcescens*, and *E. cloacae* resistant to CMD and CEZ, and the peaks of the MIC distributions of CPZ to these species were located at 3.1 to 6.25, 3.1, and 0.2 µg/ml, respectively. A notable characteristic is that CPZ has potent activity in particular against *P. aeruginosa*, for which cephalosporins used for chemotherapy are now hardly effective. Finally, CPZ has a broad spectrum of antibacterial activity against gram-positive and gram-negative microorganisms.

CPZ was effective against ampicillin-resistant *E. coli* and CB-PC- and gentamicin-resistant *P. aeruginosa* (Table 2). MIC<sub>75</sub> values of CPZ and CEZ against ampicillin-resistant *E. coli* were 8.10 and 14.5 µg/ml, respectively, and CPZ inhibited the growth of all strains at 400 µg/ml. CPZ also had high antibacterial activity against CB-PC-resistant *P. aeruginosa*, and the MIC<sub>75</sub> value of CPZ was 44.0 µg/ml. No great difference was observed between the MIC distributions of CPZ against gentamicin-resistant and -susceptible *P. aeruginosa* strains.

The effect of inoculum size on the MICs of CPZ is shown in Table 3. The MIC<sub>50</sub> and MIC<sub>90</sub> of CPZ against *E. coli* and *K. pneumoniae* fluctuated in a wider range than did those of CEZ

TABLE 2. Activity of CPZ against ampicillin (AB-PC)-resistant *E. coli* strains and CB-PC- and gentamicin (GM)-resistant *P. aeruginosa* strains

Organism	No. of organisms	Antibiotic	Drug concn (µg/ml)		
			MIC <sub>50</sub>	MIC <sub>75</sub>	MIC <sub>90</sub>
AB-PC resistant <i>E. coli</i>	50	CPZ	3.50	8.10	50.0
		CEZ	6.25	14.5	50.0
		AB-PC	>1600	>1600	>1600
CB-PC resistant <i>P. aeruginosa</i>	42	CPZ	25.0	44.0	84.0
		CB-PC	1600	>1600	>1600
GM-resistant <i>P. aeruginosa</i>	70	CPZ	7.20	17.0	80.0
		CB-PC	80.0	>100	>100
		GM	70.0	>100	>100

TABLE 3. Effect of inoculum size on MICs

Organism	No. of strains	Antibiotic	Drug concn (µg/ml)					
			MIC <sub>50</sub>			MIC <sub>90</sub>		
			10 <sup>4</sup> <sup>a</sup>	10 <sup>6</sup>	10 <sup>8</sup>	10 <sup>4</sup>	10 <sup>6</sup>	10 <sup>8</sup>
<i>E. coli</i>	200	CPZ	0.13	0.19	0.84	1.56	3.13	>100
		CEZ	1.27	1.46	4.13	5.60	6.00	50.0
<i>K. pneumoniae</i>	100	CPZ	0.18	0.21	0.84	2.95	5.20	>100
		CEZ	1.21	1.82	2.55	4.50	6.70	33.0
<i>P. aeruginosa</i>	200	CPZ	2.57	5.60	8.36	7.60	12.5	80.0
		CB-PC	28.5	35.6	57.0	90.0	94.0	>100

<sup>a</sup> Inoculum size.

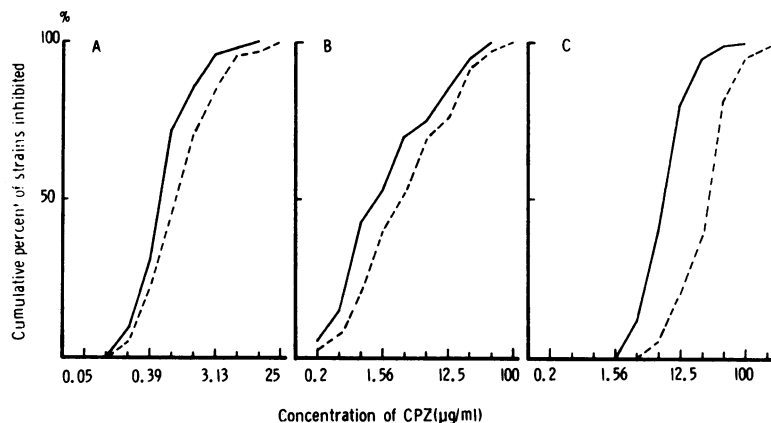


FIG. 2. Comparison of MIC and MBC values of CPZ. (A) 25 *E. coli* strains; (B) 25 *K. pneumoniae* strains; and (C) 25 *P. aeruginosa* strains.

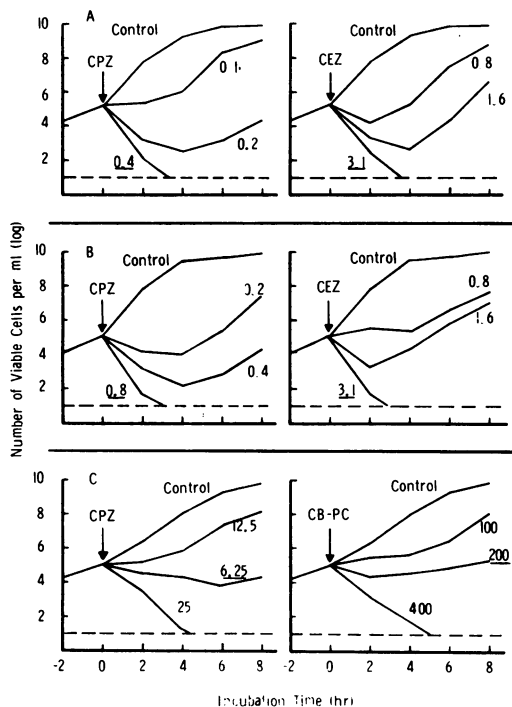


FIG. 3. Bactericidal activity of CPZ. (A) *E. coli* GN6307; (B) *K. pneumoniae* GN5558; and (C) *P. aeruginosa* GN6736. The underline indicates the MIC value (micrograms per milliliter) of each drug.

according to inoculum sizes. The inoculum size also influenced the MICs of CPZ against *P. aeruginosa* to a greater degree than it did those of CB-PC.

Addition of 10 and 40% human serum had better effect on the MICs of CPZ against *E. coli*, *P. aeruginosa*, and *S. aureus*.

**Bactericidal activity.** Comparisons of the

MICs and MBCs of CPZ are shown in Fig. 2. The MBCs of CPZ against *E. coli* and *K. pneumoniae* were two- or fourfold higher than the MICs, and the MBCs of CPZ against *P. aeruginosa* were about fourfold higher than the MICs.

The bactericidal activities of CPZ were compared with those of CEZ and CB-PC against representative strains of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* (Fig. 3). Against *E. coli* GN6307, CPZ was bactericidal at a concentration of 0.2 µg/ml, whereas CEZ was bactericidal at a concentration of 3.1 µg/ml. Against *K. pneumoniae* GN5558, these drugs were bactericidal at concentrations of 0.8 and 3.1 µg/ml, respectively, at the MIC of each drug. Against *P. aeruginosa* GN6736, CPZ was bactericidal at a concentration of 25 µg/ml, and CB-PC was bactericidal at a concentration of 400 µg/ml. The killing effects of both drugs against each of the test organisms were complete at 4 h.

**Stability to  $\beta$ -lactamase.** CPZ was stable to  $\beta$ -lactamase produced by various species of gram-negative bacilli (Table 4). CPZ was 100 to 10,000 times more stable than cephaloridine to cephalosporinases, except cephalosporinase produced by *Proteus vulgaris* (12, 15). The stability of CPZ against type I, II, and IV penicillinases mediated by R plasmids and penicillinase produced by *K. pneumoniae* was also examined (2, 7, 11, 16). CPZ was more resistant to type I and *K. pneumoniae* penicillinases than cephaloridine and 10 times more stable than penicillin G. Against type II and IV penicillinases, CPZ is considered to be a stable cephalosporin. This stability of CPZ to  $\beta$ -lactamase probably contributes to its high antibacterial activity.

## DISCUSSION

The observations summarized above show that CPZ has remarkable activity in vitro against

TABLE 4. Stability of CPZ in the presence of various  $\beta$ -lactamases

Enzyme source	Type of $\beta$ -lactamase <sup>a</sup>	Sp act <sup>b</sup>	Relative rate of hydrolysis <sup>c</sup>						
			CER	CPZ	CEZ	CET	CEX	CMD	PC-G
<i>E. coli</i> GN5482	CSase	0.24	100	<0.04	135	691	55.5	<0.04	28.7
<i>P. aeruginosa</i> GN918	CSase	0.24	100	0.04	160	480	62.9	0.04	24.8
<i>P. vulgaris</i> GN76	CSase	0.40	100	7.00	375	204	52.0	276	21.0
<i>E. cloacae</i> GN7471	CSase	3.68	100	0.80	50	402	54.0	1.70	83.1
<i>C. freundii</i> GN346	CSase	3.27	100	0.01	120	127	81.1	68.9	7.0
<i>Proteus morganii</i> GN5406	CSase	0.60	100	<0.04	73.5	242	31.0	<0.04	121
<i>E. coli</i> W3630 Rms212 <sup>+</sup>	PCase type I	2.10	18.2	12.4	7.2	7.3	<1.3	20.5	100
<i>E. coli</i> W3630 Rms213 <sup>+</sup>	PCase type II	0.23	18.8	<3.9	4.6	9.2	<2.6	11.0	100
<i>P. aeruginosa</i> ML4259 Rms139 <sup>+</sup>	PCase type IV	0.66	8.6	<0.4	<0.5	<0.5	<0.6	<0.4	100
<i>K. pneumoniae</i> GN69	PCase	0.97	15.1	13.7	2.7	2.8	<0.5	1.7	100

<sup>a</sup> CSase, Cephalosporinase; PCase, penicillinase.<sup>b</sup> Units per milligram of protein.<sup>c</sup> Hydrolysis of each substrate by PCase and CSase is expressed as the relative rate of hydrolysis, taking the absolute rate of PC-G and CER hydrolysis as 100. Abbreviations: CER, cephaloridine; CPZ, cefoperazone; CEZ, cefazolin; CET, cephalothin; CEX, cephalixin; CMD, cefamandole; PC-G, penicillin G.

a variety of gram-negative and gram-positive organisms. Whether CPZ will become a clinically useful cephalosporin will be determined by its therapeutic activity in vivo, its pharmacokinetic characteristics, and its tolerability.

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